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Detection of some heavy metals in bulk milk tank of some cows dairy herds

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Abstract

Milk is a very important component of human diet. The presence of toxic elements in milk may create significant health problems. Milk can be contaminated by different ways. One of the most important and most challenging contaminations is due to heavy metals, where its presence in fresh milk represents a risk to consumer health, as it can be the reason to the prevalence of diverse illnesses and lesions. One of the best and most efficient methods for heavy metals detection is Microwave Plasma - Atomic Emission Spectrometry. Moreover, collecting samples from Sharqia, Damietta, Monufia, Alexandria, Beheira, Faiyum, Giza, Matruh, Beni-Suef and Gharbia, storing and preparing them with different materials and solutions are also an important step to detect heavy metals. The mean concentration of Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), Chromium (Cr) and zinc (Zn) in examined milk samples were 0.0002- 0.0010.0001-0.001; 0.002-0.034; 0.0003- 0.0257; 0.00003-0.08 and 0.9- 3.46 ppm respectively, within permissible limits.

Key words: *Milk, Cd, Pb, Cu, Ni, Cr, and Zn, Microwave Plasma Atomic Emission Spectrometry, Microwave Digestion.*

Introduction

Cow's milk constitutes one of the most important sources of energy and nutrients in human diet. Milk is a perfect source of essential amino acids and vitamins. Furthermore, milk is plentiful in minerals, especially calcium, phosphorus, magnesium, potassium and chloride (**Zamberlin *et al.,* 2012**). Additionally, milk contains heavy metals, for example Cr, Fe, Mn, Zn, Cu, Co, Pb, Cd and Hg in low limits, which are toxic in high doses for human. Therefore, it is important to monitor their levels in milk considering the fact of wide range of milk consumers as children, youth, elderly, pregnant and lactating women. According to **Pereira (2014)**, the chemical characteristics of cow milk are highly dependent on the type of farming system used, the type of diet provided to the livestock, location of farm, breed, genetics, stage of lactation, environmental conditions and type of drinking water.

The contamination of milk is considered one of the main dangerous problem, Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni), and Zinc (Zn) are commonly associated with pollution and toxicity problems (**Malhat *et al.,* 2012; Mahmoudi *et al.,* 2015; Saeed *et al.,* 2017**). Heavy metals have higher density comparing to other metal elements, their average density is higher than 5g/cm^3 **(Eskandari and Pakfetrat., 2014).** One of the main problems with metals is their ability to bioaccumulate. Heavy metals enter the food chain by different ways such as sewage, waste from manufacturing activities, dust contaminated food are the usual ways. Contaminated soil also has high impact **(Eskandari and Pakfetrat, 2014 and Fallah *et al.,* 2015).** Heavy metals may enter human body by ingestion and inhalation. The intake of metals in infants mainly depends on the bioavailability of metal content in milk and milk-based foods **(Kazi *et al.,* 2009).** Number of studies have reported the prevalence of heavy metals in milk **(Ping *et al.,* 2012; Pilarczyk *et al.,* 2013; Suturović *et al.,* 2014, Ismail *et al.,* 2015 and Najarnezhad *et al.,* 2015**). Several health disorders including cells, tissues and skeletal damage, failure of lungs and kidneys, cancer of lungs and blood leukemia, and osteoporosis and anemia are associated with high level of heavy metals intake **(Ismail *et al.,* 2014; Rebelo and Coldas, 2016 and Saeed *et al.,* 2017)**. Metals and elements such as copper, Zinc, and nickel components are micronutrients and milk products are major source of these elements. If the amount of these metals and elements exceed the permissible limit, the product is considered contaminated **(Li *et al.,* 2005).** The heavy metals are responsible for many pernicious effects on human health such as saturnisme (lead contamination), immunodepression and skin diseases (zinc and copper contamination), cancer, bone disease (cadmium), increase cardiovascular diseases (chromium) (**Konuspayera *et al.,* 2009**). In order to prevent the accumulation of heavy metal in the body, Maximum Residual Limits (MRLs) by food safety authorities or organization should be followed to prevent contamination of heavy metals in foods.

The aim of this study was adopted for detection of some heavy metals (Cd, Pb, Cu, Ni, Cr, Zn) by the innovative microwave plasma atomic emission spectrometry after microwave digestion of the milk samples.

Materials and Methods

The detection of metals in milk samples were performed according to the method of the Association of Official Analytical Chemists **(AOAC, 2016).**

1- Procedure of taking milk samples:

Milk samples were collected from ten localities (Sharqia, Damietta, Menufia , Alexandria, Beheira, Faiyum, Giza, Matrouh, Beni-Suef and Gharbia) dairy farms. Fifteen samples of milk (100ml fresh) represented the collected samples from farms of each locality. The guidelines which followed up to collect good bulk tank milk samples are:

A- First hands are clean and dry. Than Identify each sample container with the patron number and date (**BMWS 2018).**

B- Agitate the milk in the bulk tank for 5 minutes before sampling.

C- Collect the sample from the top of the bulk tank and never from the outlet as milk collected from the outlet is often contaminated.

D- Use a clean sanitized dipper or sterile syringe to collect the sample.

E-Fill the sample tube 2/3 full as milk expand when frozen.

F- The sample bottles were placed immediately, in a portable ice-cooler packed with ice or in refrigerator during transportation to the laboratory and were stored at -20°C to be analyzed for heavy metals (**IDF 1985**.)

G- All the glassware had been soaked in detergent, rinsed with tap water, soaked in 15% nitric acid, rinsed with distilled water and kept in oven at 110ºC till need before use.

2- Chemicals and Standards:

All chemicals and standards are of analytical grade. Metals stock standards of Cd, Cr, Cu, Ni, Pb, and Zn were obtained from Merck, Darmstadt, Germany (1000 µg/mL).

3- Sample preparation:

Microwave digestion was used to prepare the milk samples. Ten mL of HNO_3 (33%) was added to accurately weighed 1 ml of the sample. A preloaded method for the MARS6 (CEM, Corporation, USA) microwave was used to digest the samples. Once cooled, the solution was diluted quantitatively to 10 mL using deionized water. The microwave digestion parameters were according to temperature (210°C), pressure (800), time (ramp-Hold , 21-15min), power 400-1800, vessels (Easy prep full starter set, p/T control. All measurements of this method were performed using the innovative Agilent microwave plasma atomic emission spectrometry model 4200 MP-AES with nitrogen gas plasma supplied via an Agilent 4107 nitrogen generator according to the following parameters **(Cauduro , 2013).**

Microwave Plasma Atomic Emission Parameters

Parameter	Value
Replicates	3
Pump rate	15 rpm
Sample uptake delay	15 seconds
Rinse time	30 seconds
Stabilization time	15 seconds
Fast Pump during Uptake and Rinse	On (80rpm)
Nebulizer	One Neb
Spray chamber	Double pass cyclonic
Autosampler	Agilent SPS 3
Sample pump tubing	Orange/green
Waste pump tubing	Blue/blue

Moreover, heavy metals measurements to determine their residues should follow the following wavelengths (nm) and calibration range (µg/mL) corresponding to each element

Element	Wavelength (nm)	Calibration Range (µg/mL)	Correlation Coefficient (R^2)
Cadmium (Cd)	226.502	0 – 2.500	0.99995
Lead (pb)	405.781	0 – 2.500	0.99991
Copper (Cu)	324.754	0 – 2.500	0.99935
Nickel (Ni)	352.424	0 – 2.000	0.9999
Chromium (Cr)	425.433	0 – 2.000	0.9999
Zinc (Zn)	213.857	0 – 1.500	0.99769

4- Preparation of standards of heavy metals:

The heavy metals selected for this study were Cd, Pb, Cu, Ni, Cr and Zn. In each case of the selected metals, three different concentrations were made to calibrate the microwave plasma atomic emission spectrometry. These concentrations were as follows: 0.5 ppm, 1.0 ppm and 2.0 ppm. The resultant calibration curve of well-prepared standard concentrations was represented by linear curve by using microwave plasma atomic emission spectrometry (Table 2).

Statistical analysis:

Data were statistically evaluated by one-way analysis of variance (ANOVA). All statistical analyzes were done using the statistical package for social sciences (SPSS 16.0) program (mean ±SE).

Results and Discussion

Environmental risk factors possess a substantial contribution in the burden of diseases both globally and nationally. Due to high stability in the nature and bioaccumulation of these pollutants, various public health concerns are identified. Plants are able to absorb heavy metals from the soil, such plants with high level of heavy metals contents can be used as animal feed, this in turn, results in contamination of animal products such as milk. Consequently, metal residues in milk may possess further adverse effects on consumer's health and in particular retardation mental among children and infants. In industrial areas, milk pollution is important due to possibility of heavy metal contamination linked to industrial units. It should be noted that milk is not the only source of absorption of these toxic metals, Therefore, the present research might be considered as an important challenge.

Table (1). The limit of detection (LOD) and limit of quantification (LOQ) different metals in milk

Element	LOD (µg/L)	LOQ (µg/L)
Cadmium (Cd)	2.100 $\times 10^3$	7.000 $\times 10^3$
Lead (pb)	3.300 $\times 10^3$	0.011
Copper (Cu)	0.700 $\times 10^3$	2.333 $\times 10^3$
Nickel (Ni)	0.900 $\times 10^3$	3.000 $\times 10^3$
Chromium (Cr)	0.100 $\times 10^3$	0.333 $\times 10^3$
Zinc (Zn)	4.500 $\times 10^3$	0.015

LOD: The Limit of Detection (=3X standard deviation of 10 measurements in blank matrix).
LOQ: The Limit of Quantification (= 3XLOD).

Table (2). Mean ± SE values (ppm) of recovery for heavy metals in milk (n =4).

Conc.	Cadmium	Lead	Copper	Nickel	Chromium	Zinc
0.5	96.09±0.45	95.75±0.66	95.54±0.49	96.04±0.30	95.58±0.73	97.29±0.31
1	95.90±0.74	94.75±0.54	96.83±0.63	95.21±1.07	95.76±0.69	94.56±0.35
2	97.12±0.49	96.46±0.37	97.54±0.28	96.30±0.77	96.83±0.88	96.96±0.35

Table (3). Some critical levels of observed metals published by WHO⁄FAO and Food and Nutrition Board.

Element	Criticallevels	Reference
Cadmium	0.02(µg/g)	**Codex Alimentarius Commission, (2004)**
Lead	0.02 (µg/g)	**Codex Alimentarius Commission (2011)**
Copper	0.01 (µg/g)	**IDF (1979)**
Nickel	0.2(µg/g)	**Codex Alimentarius Commission, (2013)**
Chromium	0.3(µg/g)	**by GB/T 5009.123(2003)**
Zinc	121 (µg/g)	**Ijaz, *et al* (2017)**

Heavy metals concentrations in the milk samples from 10 different districts of Egypt are presented in Table (4). The statistical analysis showed significant differences in the concentrations of various heavy metals (Cd, Pb, Cu, Ni, Cr and Zn) within different districts.

Table (4). The mean concentration of some heavy metals in cow milk samples collected from different localities (ppm=µg/g) n=15

Localities	Cd	Pb	Cu	Ni	Cr	Zn
Sharkia	0.0004 ± 0.0002	0.0013 ± 0.0006	0.0057 ± 0.0016	0.0167 ± 0.0062	0.0800 ± 0.0464	1.1967 ± 0.3623
Demitta	0.0004 ± 0.0002	0.0010 ± 0.0004	0.0057 ± 0.0016	0.0167 ± 0.0062	0.0800 ± 0.0464	1.1967 ± 0.3623
Menufea	0.0003 ± 0.0002	0.0010 ± 0.0004	0.0020 ± 0.0004	0.0257 ± 0.0157	0.0010 ± 0.0007	0.9267 ± 0.4247
Alexandria	0.0002 ± 0.0001	0.0001 ± 0.0001	0.0030 ± 0.0008	0.0023 ± 0.0010	0.00033 ± 0.00024	1.5700 ± 0.9080
Beheria	0.0004 ± 0.0002	0.0002 ± 0.0001	0.0080 ± 0.0043	0.0003 ± 0.0002	0.00003 ± 0.00002	3.0300 ± 1.2073
Fayium	0.0003 ± 0.0002	0.0001 ± 0.0000	0.0080 ± 0.0043	0.0137 ± 0.0093	0.00033 ± 0.00024	2.8267 ± 1.0099
Giza	0.0004 ± 0.0002	0.0005 ± 0.0002	0.0110 ± 0.0035	0.0013 ± 0.0006	0.00003 ± 0.00002	3.4600 ± 0.6201
Matrouh	0.0010 ± 0.0004	0.0007 ± 0.0005	0.0027 ± 0.0008	0.0017 ± 0.0008	0.00010 ± 0.00004	2.4400 ± 1.0498
Beni-suef	0.0006 ± 0.0002	0.0041 ± 0.0021	0.0343 ± 0.0232	0.0003 ± 0.0002	0.00077 ± 0.00044	2.6767 ± 1.1587
Gharbia	0.0006 ± 0.0002	0.0014 ± 0.0006	0.0027 ± 0.0012	0.0007 ± 0.0002	0.0024 ± 0.0016	3.3567 ± 0.5479

Data represented as mean ± SE

Cadmium (Cd) toxicity in humans may lead to kidney failure as well as liver and skeletal disorders **(Zaidan *et al.,* 2013).** Milk usually contain very low concentration of Cd except when dairy animals consumed contaminated feeds and water. Moreover, contamination during storage, marketing and leaching from containers may be considered as a source of Cd in milk. A study conducted by **Rodríguez *et al.* (1999)** discussed that Cd in cow milk samples was higher when protein content in milk increased and mentioned that in other study (**Mata *et al.,* 1995)** Cd content was associated with the protein fraction (casein fraction) in milk.

In the present study, mean cadmium concentrations in milk was in the ranges 0.0002- 0.001 ppm Table (4).The presence of Cd in milk may be due to either natural or anthropogenic origins (fertilizers and atmospheric deposition in soils). It is considering to be the most important contaminant in modern times **(Arafa *et al.,* 2014)**. The maximum limit for Cd in milk reported by (**CAC, 2004**) is 0.02 ppm which is high as compared with the obtained results in the present study. The mean levels of Cd reported in bovine milk from Egypt by **Enb *et al.* (2009)** and from Nigeria by **Ogabiela *et al.* (2011)** are 0.086 µg/g and 0.131 µg/g, respectively, which are much higher as compared to the current findings. However, a very low Cd level is reported in Spain (0.0004 µg/g) and Korea (0.002 µg/g) by **Sola and Navarro (2009)** and **Khan *et al.* (2014),** respectively, which agreed with the obtained results in the current study.

Lead (Pb) is one of the most toxic heavy metals and its level in milk is increasing day by day due to the uncontrolled urbanization and industrialization (**Swarup *et al.,* 2005).** Milk samples in this study were found to contain Pb concentrations in the range of 0.0007-

0.001ppm. A comparison of our results with this standard limit showed milk samples were within the permissible limit (0.02 µg/g) recommended by **CAC, (2011)** and (**FAO/WHO, 2012)**.

There are a number of studies from Egypt, concerning the Pb contamination in milk, **El-Sayed *et al.* (2011), Abd-El Aal *et al.* (2012), Hassan and El- Shahat (2012)** and **Arafa *et al.* (2014)** determined Pb concentrations in milk taken from different regions in Egypt as 0.071–1.53, 0.04–0.08, 1.850–4.404 and 0.044 –0.751ppm respectively, which are much higher as compared to present study. The mean level of Pb reported in cow milk samples from Iran (**Najarnezhad and Akbarabadi, 2013)** was 0.012 µg/g which is slightly higher as compared to the present study and agreed Pb level in milk with that reported in Korea (0.004 µg/g) by **Khan *et al.* (2014).** The presence of Pb in milk may be due to environmental sources (atmospheric deposition, waste disposal, vehicle exhausts, urban effluent etc).

Copper (Cu) is an essential element for skin and blood vessel's strength, for the production of myelin and hemoglobin and the proper functioning of enzymatic systems (**Osredkar and Susta 2011).** However, the excessive intake of Cu may lead to immunity disorders, dermatitis, impaired nervous system, gastrointestinal and neurological problems (**Barn *et al.,* 2014**). The results of the Cu concentration measurements are shown in Table (4) with certain variations of these element in the milk samples collected from different regions. Concentration of Cu was observed in the milk samples collected from the farms in the rang 0.002-0.034 ppm, while permissible limits for Cu is 0.01 µg/g according **to IDF (1979)**. In Egypt, **Malhat *et al.* (2012) and Amir *et al.* (2017)** reported 1.451 µg/g Cu in milk samples this value is much higher as compared to the findings of the present work . **Amir *et al.* (2017)** reported the levels of Cu in the milk ranged 0.018 – 0.141 µg/g.

Nickel (Ni) being a cofactor for a number of hormones and enzymes is considered as essential element for humans, it enhances insulin activity (**Belitz *et al.,* 2004**). Nickel has a great affinity for cellular structures such as chromosomes and ion channels. However, excessive nickel in tissues is pro-oxidant (damaging chromosomes and other cell components) and alters hormone and enzyme activities, movement of ions through membranes and immune function. Under some conditions, large amounts of nickel may precipitate magnesium deficiency or cause accumulation of iron or zinc (**Kenney, 2010).** Also**,** nickel has negative effects on the structure and function of the testis, seminal vesicles, prostate gland and adverse effect on spermatozoa (**Forgacs *et al.,* 2001**). In addition to that, long term exposure to nickel causes decrease body weight, skin Irritation (**Hamidi Ravari and Daneshpajooh 2009)**, heart and liver damage (**Kusal and Verginia 2007**). Nickel concentration in the present study was found in range of 0.0007-0.00257 ppm within permissible limit (0.2µg/g**) of CAC (2013**). Our results were agreed with **Enb *et al.* (2009)** determined levels of nickel 0.002-0.009 mg/kg in milk from area of Giza **-**Egypt.

Chromium is an important mineral through which the body must have to function properly. It facilitates the action of insulin as well as helps the metabolism and storage of carbohydrate, fat, protein and also help controls blood cholesterol levels (**Ikem and Egilla 2008)**. The body stores chromium in the blood and in the hair. The acceptable daily intake of chromium is 50- 200µg/day. Low level exposure to chromium can cause skin irritation, and ulceration. Long term exposure can cause kidney and liver damage as well as circulatory and nerve tissue problems (**Dayan and Paine, 2001 and Lenntech, 2004)** and it can hamper the function of DNA and can cause genotoxicity **(Cohen *et al.,* 1993).** The major concern about chromium is that it has carcinogenic behavior in humans **(Baruthio, 1992).** The average chromium content was found in the present study was

0.00003- 0.08 ppm which is lower that the permissible limit (200µg/l).

The chromium results of this research are closely related to the values reported by **Hasan and Arzu (2012)** were 0.013 - 0.075 mg⁄ kg. while, **Enb *et al.,* (2009)** determined levels of chromium 0.028 - 0.066 mg/kg in milk from Giza **-**Egypt. **Ogabiela *et al.* (2011)** found that Cr content of examined samples were 0.001-7.728mg/l. According to the Table (4), the amount of chromium in Menufea was 0.001 ppm, which accordance with the values determined by **Ogabiela *et al.,* (2011)** in milk.

Zinc has a lot of functions in the human body. It is required for the structure and activity of more than 300 enzymes, for nucleic acid and protein synthesis(DNA)(**Beigi and Maverakis, 2015 and Tariba *et al.,* 2017),** cellular differentiation and replication, glucose use and insulin secretion, exerts regulatory actions in various aspects of hormone metabolism. Zinc plays an important role in the immune system, reproduction, (**Nada *et al.,* 2010**). Zinc deficiency in animals causes serious disorders, while excess intake of zinc through food, water or dietary supplements can affect health. If large doses of zinc by mouth even for a short time, stomach cramps, nausea and vomiting may occur. Ingesting high levels of zinc for several months may cause anemia by suppresses copper and iron absorption, which results in decrease in erythrocyte (**Belitz *et al.,* 2004**), damage of pancreas and decrease levels of high – density lipoprotein (HDL) cholesterol **(Tassew *et al.,* 2014).** Zinc recorded in this study was found to be 0.9267ppm as the least concentration while 3.460 ppm as the highest concentration analyzed and all these are below the permissible limit of zinc which is (121mg/kg) **(Ijaz *et al.,* 2017**). This result are in agreement with **Metin (2010**) who reported the concentration of Zinc in milk was 3.5 mg⁄ kg, while it was higher than that reported by **Arafa *et al.,* (2014)** while lower level were reported by (**Licata *et al.,* 2004 and Naqib Ullah *et al.,* 2015).**

The mean zinc concentration of samples in Alexandria (table 4) was found to be1.57ppm, this value coincides with the levels obtained by **Imam *et al.* (2017**). Zinc obtained was found 1.5mg/kg.

Conclusion and Recommendation

The study was done to determine the concentration of heavy metals and compare it against the limits which are set by the World Health Organization (WHO). This study revealed concentrations of some heavy metals, which were below permissible limits according to Codex Alimentations Commission. Therefore, it is recommended that a more specific confirmatory test like seasonal variations in amounts of the metals, since pattern of animal feeding is evidently different within the seasons. Furthermore, examining amount of the selected metals in animal feeds and even in soil and water resources of the study region could help better explanation of the results which warrants further studies.

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